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Applicant: Marc LEMAIRE  
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Title: Use of N2O in the treatment of post-ischemic brain cell deterioration  
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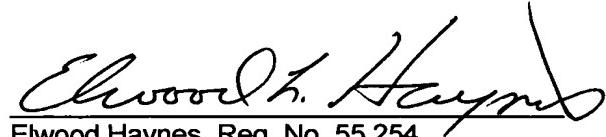
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Dear Sir:

Applicant respectfully submits herewith the English translation of the application for priority FR 0350002 in connection with the above-identified application.

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Respectfully submitted,

  
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Use of N<sub>2</sub>O in the treatment of post-ischaemic brain  
cell deterioration

The invention relates to the use of nitrous oxide (N<sub>2</sub>O) 5 or of an N<sub>2</sub>O donor for producing all or part of a medicinal product intended to treat or prevent post-ischaemic brain cell deterioration, in particular deterioration subsequent to a stroke, especially all or part of an inhalable gaseous medicinal product, in 10 humans or animals.

In cerebral ischaemia subsequent to a stroke, and in strokes in general, a functional alteration of many neurotransmission systems is usually noted from a 15 neurochemical point of view, in particular an increase in the release of glutamate, the excitotoxicity and contribution of which to neuronal death are known, as recalled by *Dirnagl et al., Trends Neurosci, 22: 391, 1999.*

20 Moreover, from a functional point of view, in the case of global ischaemia in the rat, an increase is observed in locomotor activity, in particular described by *Wang and Corbett, Brain Res., 533: 78, 1990; Baldwin et al., Neurodegeneration 2: 139, 1993*, the development of which is generally attributed to an alteration in cognitive functions of spatial recognition rather than to an alteration in sensory-motor functions.

30 As a result, a potential therapeutic role for ionotropic and metabotropic glutameric receptor antagonists have been suspected, in particular by *Chazot, Curr Opin Invest Drugs 1: 370, 2000; Drian et al., Neurochem Int 38: 509, 2001.*

35 It also appears that the deleterious effects of known cerebral ischaemias appear to involve localized ischaemias which are thought to be caused by glutameric excitotoxicity.

- In fact, the therapeutic potential of glutameric receptor antagonists is often put forward in the treatment of neuropathologies of excitotoxic origin, in particular cerebral ischaemia, as described by *Dirnagl et al.*, *Trends Neurosci* 22: 391, 1999, and productive disorders, as described by *Benes*, *Brain Res. Review* 31: 251, 2000.
- 10 However, the physiology of glutameric receptors is complex and it appears that the high affinity antagonists may also exhibit neurotoxic properties, according to *Burns et al.*, *Psychopharmacology* 115: 516, 1994.
- 15 Thus, a potential therapeutic advantage of low affinity antagonists, in particular for NMDA, has recently been proposed by *Palmer and Widzowski*, *Amino acids* 19: 151, 2000.
- 20 Moreover, document WO-A-02/09731 is also known, which relates to the use of CO, optionally supplemented with another gas, for treating inflammations of the upper respiratory tracts or of the bronchi. That document 25 therefore targets the treatment of pathologies such as asthma, cystic fibrosis, pneumopathies or the like.
- 30 In addition, document EP-A-861672 teaches a method of treatment which can be used in emergency situations by administering various gases. However, this does not relate to post-ischaemic brain cell degradations subsequent to strokes.
- 35 Finally, document FR-A-2812545 teaches a combination of gas and active product intended to treat or prevent pain. The active product is an analgesic, an anti-inflammatory, an anti-pyretic or the like.

To date, no effective medical product therefore exists for preventing or treating, at least partially, post-ischaemic brain cell degradation subsequent to strokes.

5 The present invention falls within this context, and aims to provide all or part of a medicinal product which can be used for preventing, decreasing or treating any post-ischaemic brain cell deterioration, in particular subsequent to a stroke, in humans or  
10 animals.

The invention therefore relates to the use of nitrous oxide ( $N_2O$ ) or of an  $N_2O$  donor for producing all or part of a medicinal product intended to treat, minimize or  
15 prevent post-ischaemic brain cell deterioration.

Depending on the case, the use of the invention may comprise one or more of the following technical characteristics:

20 - all or part of the gaseous medicinal product is in inhalable form;

25 - the post-ischaemic brain deterioration results in or is subsequent to a stroke;

- the nitrous oxide ( $N_2O$ ) or the nitrous oxide donor is in gaseous form or is included in a gas or a mixture of gases;

30 - the medicinal product contains an effective proportion of nitrous oxide ( $N_2O$ ) or of  $N_2O$  donor;

35 - the medicinal product also contains xenon or a xenon donor, the xenon or the xenon donor being in gaseous form or being included in a gas or a mixture of gases;

- the medicinal product contains an effective proportion of xenon or of a xenon donor;
- the medicinal product also contains at least one other gaseous compound chosen from oxygen, nitrogen or argon, preferably nitrogen and oxygen;
- the medicinal product contains an amount which is less than 60% by volume of xenon or of xenon donor, preferably less than or equal to 50% by volume;
- the medicinal product contains an amount ranging up to approximately 80% by volume of N<sub>2</sub>O or of N<sub>2</sub>O donor, preferably up to 75% of N<sub>2</sub>O;
- the medicinal product contains from 19 to 25% by volume of oxygen and, optionally, of nitrogen.

The invention therefore also relates to an inhalable medicinal product with neuroprotective action in the brain, containing an effective amount of nitrous oxide (N<sub>2</sub>O) or of a donor of such a compound, in particular intended to treat, minimize or prevent post-ischaemic brain cell deterioration.

According to the case, the medicinal product of the invention may comprise one or more of the following technical characteristics:

- it contains an amount ranging up to 80% by volume of gaseous N<sub>2</sub>O;
- it contains an effective amount of nitrous oxide (N<sub>2</sub>O) or of a donor of such a compound;
- it contains an effective amount of xenon or of a donor of such a compound;

- it contains an amount which is less than 60% by volume of xenon;
- it also contains from 19 to 25% by volume of 5 oxygen and, optionally, of nitrogen.

The idea on which the present invention is based is to take advantage of the NMDA receptor antagonist properties of gaseous N<sub>2</sub>O, optionally supplemented with 10 xenon, for their neuroprotective nature, in prevention or treatment of post-ischaemic pathologies subsequent to strokes.

In fact, recent studies, carried out *in vitro*, have 15 shown that N<sub>2</sub>O, and also xenon, can potentially behave like low-affinity antagonists of glutameric receptors for N-methyl-D-aspartate, NMDA (*Franks et al., Nature 396: 324, 1998; Jevtovic-Todorovic et al., Nature Med. 4: 460, 199; Yamakura and Harris, Anesthesiology, 20 20008*).

Based on these observations, experiments were carried out in the context of the present invention, with the aim of determining the neuroprotective effects of N<sub>2</sub>O 25 and of xenon, on neuronal death induced by transient cerebral ischaemia in rats.

In order to demonstrate the beneficial effect of administering N<sub>2</sub>O or xenon on brain cells subsequent to 30 cerebral ischaemia, adult Sprague-Dawley rats weighing 350 g were subjected to the following experimental protocol.

On day 1, focal ischaemia was induced in each of the 35 rats by middle cerebral artery occlusion (MCAO), for a period of 1 h 30 minutes.

The transient focal cerebral ischaemia by MCAO is obtained conventionally by introducing a flexible nylon

thread 1, represented diagrammatically in Figure 1 (length 6.5 mm, diameter 180  $\mu\text{m}$ ), a portion 2 of the proximal end of which has a diameter greater than that of the thread (length 3 mm, diameter 380  $\mu\text{m}$ ), into the 5 vascular system of the rat, as far as the region of the ipsilateral hemisphere so as to cause an embolism therein, i.e. an ischaemia.

Next, the rats are reperfused for 10 to 20 minutes, and 10 are then made to inhale several mixtures of gases, namely:

- mixture No. 1: air (control)
- mixture No. 2:  $\text{N}_2\text{O}$  (75% vol), the remainder being 15 oxygen (25%)
- mixture No. 3: xenon (50% vol), the remainder being oxygen (20 to 25%) and nitrogen (30 to 25%), respectively
- mixture No. 4: xenon (75% vol), the remainder 20 being oxygen (25%).

On day 2, i.e. 24 hours after reperfusion, the rats are killed, the brains are recovered and frozen, and thin sections 40  $\mu\text{m}$  thick are cut and then stained with 25 cresyl violet, as shown in Figure 5.

The volume of neuronal death is calculated, from the sections obtained after staining, in a conventional manner using an appropriate, commercially available 30 conventional program.

In fact, as shown diagrammatically in Figure 2, the cerebral ischaemia engenders, in general, in 24 hours, an infarction in the region which has been subjected to 35 ischaemia (penumbra), leading to neuronal death in the brain cells present in a considerable portion of this region.

The results obtained during these measurements have been recorded in Figures 3a to 3d, which make it possible to visualize the post-cerebral ischaemia neuroprotective effect of mixtures No. 2 to 4 above, in 5 comparison with mixture No. 1 (air) which serves as a control.

Thus, Figure 3a clearly shows that inhalation by the rats of nitrous oxide ( $N_2O$ ) or of xenon (Xe) subsequent 10 to an ischaemia makes it possible to considerably reduce the total volume of infarction, since a decrease in this volume of approximately 50% can be achieved in the case of inhalation of mixtures No. 2 and No. 3 instead of air (mixture No. 1 acting as control), and 15 of approximately 30% when mixture No. 4 is inhaled.

In this respect, it will also be noted that inhalation of 50% by volume of xenon (mixture No. 3) is more effective than inhalation of a higher dose of xenon, 20 namely 75% (mixture No. 4), which implies that the most effective dose appears to be closer to 50% than to 75% with regard to xenon.

Figures 3b to 3d confirm the results of Figure 3a, 25 since they make it possible to observe that inhalation of xenon or of  $N_2O$  makes it possible to decrease, respectively, the post-ischaemic volume of cortical infarction (Fig. 3b), the post-ischaemic volume of striatal infarction (Fig. 3c) and the post-ischaemic 30 volume of oedema (Fig. 3d), compared to inhalation of air (control = mixture No. 1).

Based on this observation, complementary examinations were carried out in order to determine the neurotoxic 35 effects of the xenon and of the nitrous oxide ( $N_2O$ ), at various amounts, compared to air, on brain receptors of the NMDA type.

The results of these examinations are reported in Figure 4, which clearly shows that the administration of xenon or of nitrous oxide engenders a smaller volume (in  $\text{mm}^3$ ) of deteriorated NMDA receptors than the 5 control (air), this being with the nitrous oxide given at a dose of 50% or 75% by volume (remainder = 25% of  $\text{O}_2$ ) and the xenon given at a dose of 50% or 75% (remainder = mixture of 25% of  $\text{O}_2$  + 25% of  $\text{N}_2$ , or, respectively, 25% of  $\text{O}_2$ ).

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However, a neurotoxic effect which is variable according to the dose administered thus emerges, leading to the observations that  $\text{N}_2\text{O}$  at 75% and xenon at 50% by volume are more neuroprotective than  $\text{N}_2\text{O}$  at a 15 dose of 50% and xenon at a dose of 75%.

In other words, these data confirm that administration by inhalation of  $\text{N}_2\text{O}$  at a dose of 75% by volume (or less) or of xenon at a dose of 50% by volume (or less) 20 engenders a neuroprotective action with respect to cerebral ischaemia and other similar excitotoxic diseases.

The inhalable medicinal product according to the 25 invention is packaged in pressurized gas containers, such as gas bottles, and is dispensed to the patient via an appropriate system for administering gas, equipped with a breathing mask, a tracheal catheter, or the like.

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Consequently, in the context of the invention, the use of gaseous nitrous oxide ( $\text{N}_2\text{O}$ ) will be preferred to that of xenon, for producing a medicinal product intended to treat, minimize or prevent post-ischaemic 35 brain cell deteriorations, such as strokes.

Claims

1. Use of nitrous oxide ( $N_2O$ ) or of an  $N_2O$  donor, for producing all or part of a medicinal product intended  
5 to treat, minimize or prevent post-ischaemic brain cell deterioration.
2. Use according to Claim 1, wherein all or part of the gaseous medicinal product is in inhalable form.  
10
3. Use according to Claim 1, wherein the post-ischaemic brain deterioration results in or is subsequent to a stroke.  
15
4. Use according to Claim 1, wherein the medicinal product also contains xenon or a xenon donor, the xenon or the xenon donor being in gaseous form or being included in a gas or mixture of gases.  
20
5. Use according to Claim 1, wherein the nitrous oxide ( $N_2O$ ) or the nitrous oxide donor is in gaseous form or is included in a gas or in a mixture of gases.  
25
6. Use according to Claim 1, wherein the medicinal product contains an effective proportion of nitrous oxide ( $N_2O$ ) and/or of xenon or of an  $N_2O$  or xenon donor.  
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7. Use according to Claim 1, wherein the medicinal product also contains at least one other gaseous compound chosen from oxygen, nitrogen or argon, preferably nitrogen and oxygen.  
35
8. Use according to Claim 1, wherein the medicinal product contains an amount which is less than 60% by volume of xenon or of xenon donor, preferably less than or equal to 50% by volume.

9. Use according to Claim 1, wherein the medicinal product contains an amount ranging up to 80% by volume of N<sub>2</sub>O or of N<sub>2</sub>O donor, preferably up to 75% of N<sub>2</sub>O.
- 5 10. Use according to Claim 1, wherein the medicinal product contains from 19 to 25% by volume of oxygen and, optionally, of nitrogen.
- 10 11. Inhalable medicinal product with neuroprotective action in the brain, containing an effective amount of nitrous oxide (N<sub>2</sub>O) or of a donor of such a compound.
- 15 12. Medicinal product according to Claim 11, further containing an amount ranging up to 80% by volume of N<sub>2</sub>O.
- 20 13. Medicinal product according to Claim 11, further containing xenon or a donor of such a compound, preferably in an amount which is less than 60% by volume of xenon.
14. Medicinal product according to Claim 11, further containing from 19 to 25% by volume of oxygen and, optionally, of nitrogen.
- 25 15. Pressurized gas container containing a medicinal product according to Claim 11, in particular a gas bottle.
- 30 16. Method for treating, minimizing or preventing post-ischaemic brain cell deterioration with a patient comprising the step of :  
(a) administrating an therapeutically-effective amount of a medicinal product containing nitrous oxide (N<sub>2</sub>O) or of an N<sub>2</sub>O-donor to the patient.

17. Method according to Claim 16, wherein at least one part of the gaseous medicinal product is in inhalable form.

5 18. Method according to Claim 16, wherein the post-ischaemic brain deterioration results in or is subsequent to a stroke.

10 19. Method according to Claim 16, wherein the medicinal product also contains xenon or a xenon donor, the xenon or the xenon donor being in gaseous form or being included in a gas or mixture of gases.

15 20. Method according to Claim 16, wherein the nitrous oxide ( $N_2O$ ) or the nitrous oxide donor is in gaseous form or is included in a gas or in a mixture of gases.

20 21. Method according to Claim 16, wherein the medicinal product also contains at least one other gaseous compound chosen from oxygen, nitrogen or argon, preferably nitrogen and oxygen.



PATENT APPLICATION  
for:

"Use of N<sub>2</sub>O in the treatment of post-ischaemic brain  
cell deterioration"

in the name of

L'AIR LIQUIDE SANTE (INTERNATIONAL)

Invention by: M. Lemaire

- - - - -

ABSTRACT

The invention relates to the use of nitrous oxide (N<sub>2</sub>O) or of a donor of such a compound, for producing all or part of a medicinal product, preferably in inhalable form, intended to treat, minimize or prevent post-ischaemic brain cell deterioration, in particular subsequent to a stroke. The medicinal product contains an amount ranging up to 80% by volume of N<sub>2</sub>O. The medicinal product also contains from 19 to 25% by volume of oxygen and, optionally, of nitrogen and/or an amount which is less than 60% by volume of xenon.

No figure